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Interaction of Mefenamic Acid with Cobalt(II) Ions in Aqueous Media: Evaluation via Classic and Response Surface Methods

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Mefenamic acid reacts with cobalt(II) ions in aqueous alkaline medium to form a highly stable deep brown complex at ambient temperature. The complexation process was optimized in terms of pH, temperature, agitation rate, and contact time using classical studies. A response surface method based on Box-Behnken design was used to statistically model the complexation reaction and investigate factor effects along with their interactions. A quadratic model was developed using experimental data with a correlation coefficient of 0.9609. Numerical optimization was performed to achieve the optimum solutions of factor combinations. The results of the classical investigation and statistical method were in close agreement with each other, while the advantages of the modern experimental design method over conventional one-factor-at-a-time studies were revealed.

Key Words: Mefenamic acid; cobalt; Box-Behnken; complexation; classical method

Introduction

Mefenamic acid [(2,3-dimethyl diphenyl) amino-2-carboxylic acid] belongs to the acidic, nonsteroidal, and anti-inflammatory drugs (NSAIDs).[1] It is used for the relief of short-term moderate pain lasting less than 1 week, such as muscular aches and pains, menstrual cramps, headaches, and dental pain. The anti-inflammatory activity of NSAIDs can be attributed to inhibition of the conversion of arachidonic acid to prostaglandins, which are mediators for the inflammation. Modern studies have revealed that in addition to arthritis and pain, cancer and neurodegenerative diseases such as Alzheimer's disease could be potentially treated with NSAIDs due to the inhibition effect on cox-2, which is a recently characterized cyclooxygenase.[2,3]

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Synthesis and investigation of metal complexes with active pharmaceuticals in which the drug molecules play a role of ligand have been regarded as a research domain of increasing interest for inorganic, pharmaceutical, and medicinal chemistry. These studies have attracted much attention as an approach to new drug development. It has been revealed that metal complexes of anti-inflammatory drugs have lower toxicity and higher pharmaceutical effect compared to the free drug owing to the inhibition of metal complexation with other important biological compounds.[4−7] Carboxylates are among the most ubiquitous compounds and the increasing interest in their complexes can be attributed to significantly important biological properties.[8,9] The chemical structure of mefenamic acid, which possesses a carboxylate group, is promising for chelation with metal ions. The active binding site of mefenamic acid with metal ions is the oxygen atom of the carboxyl group.[10] Moreover, the high molecular weight of this reagent provides supporting evidence of its suitability for spectrophotometric methods. Complexation of mefenamic acid with alkaline metals has been reported,[10] while there has been no report of complexation between mefenamic acid and transition metals.

The importance of cobalt as a transitive element can be attributed to its various applications, particularly in the pharmaceutical domain. Various cobalt complexes have been developed that are able to inhibit the protein tyrosine kinases selectivity. These complexes are useful in the treatment of various diseases.[11]

Nowadays, optimization techniques are called into play every day in questions of industrial planning, resource allocation, scheduling, laboratory processes, etc. Classic optimization can be done by varying any of the process parameters and keeping the other parameters constant. When multiple variables are involved, it becomes difficult to study the system using the common approach of varying only one factor at a time while holding the others constant. The new statistical designs consider all factors simultaneously and hence provide the possibility for evaluation of all the effects at once. Modern experimental designs have been regarded as the most favorable techniques in covering a wide area of practical statistics and obtain unambiguous results with the least expense.

Response surface methods (RSMs) have been designed for factors with more than 3 levels in which quadratic models can be established. The main objective is to find a desirable location in the design space. This could be a maximum, a minimum, or an area where the response is stable over a range of the factors. After clarifying the goal, the next step is to figure out which responses will be measured and how to measure them. Quantifiable response is one of the most important steps in a prosperous design of experiments (DOEs). The most popular response surface methodologies are central composite, Box-Behnken, and Doehlert designs.[12−15]

Box-Behnken is a response surface design, particularly made to require only 3 levels, coded as -1, 0, and +1. Box-Behnken designs are formed by combining 2-level factorial designs with incomplete block designs.[16,17] This procedure creates designs with desirable statistical properties but, most importantly, with only a fraction of the trials required for a 3-level factorial. Because there are only 3 levels, the quadratic model is appropriate. The number of experiments required for Box-Behnken design can be calculated according to $N = k^2 + k + c_p$, where k is the factor number and c_p is the replicate number of the central point. If viewed as a cube (Figure 1), it consists of a central point and the middle points of the edges.

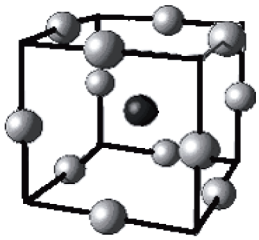

Figure 1. Cube derived Box-Behnken design.

Response surface methods have been applied most popularly in recent years and works based on the application of RSM in different branches of chemical, biochemical, and chemical engineering fields have been reported.[18−23]

The objective of this work was firstly to investigate the effective variables, namely pH, temperature, agitation rate, and contact time, on mefenamic acid-cobalt(II) complex formation and secondly to use computer-aided design methodology for achieving the optimum conditions via fewer experimental trials, confirming the results of the classical approach and modeling of the process. The experimental data were obtained using spectrophotometry.

Experimental

Chemical substances

Mefenamic acid was purchased from RAZAC Pharmaceutical Co. (Iran) as a white powder with a melting point of 230.5 °C and 99.8% purity. It was odorless and sparingly soluble in water (40 mg/L at 25 °C and 80 mg/L at 37 °C) but significantly soluble in alkaline media (10 g/L at pH 7.1). The pK_a value for a pure reagent was 4.2. The chemical structure of mefenamic acid is shown in Figure 2.

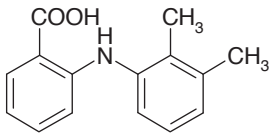

Figure 2. Chemical structure of mefenamic acid.

All chemicals and solvents, namely methanol, cobalt(II) chloride hexahydrate, tri-ethanol amine, acetic acid, sodium acetate, and nitric acid, were of analytical grade and obtained from Merck Chemical Company. Deionized water was used for preparation of all solutions.

Practical procedure

Preparation of complex

Mefenamic acid (10^{-2} mol/L) was dissolved in dimethylformamide (DMF) solution. The resulting solution was added to a 2 mL portion of 100 μg/mL aqueous cobalt(II) solution in a 20 mL test tube with constant stirring at 180 rpm. The acidity of initial metal solutions was adjusted to various pH values (5-10). Triethanolamine was titrated with 10^{-2} mol/L nitric acid to produce buffers required for adjusting the pH of alkaline media. The pH of acidic solutions was adjusted using acetate buffer. After the desired

contact time at room temperature, the deep brown complex was transferred to a 10 mL volumetric flask and diluted to the total volume with distilled water. The absorbance was measured by a CECIL 9200 double-beam spectrophotometer using aqueous cobalt(II) solution as reference at 560 nm (λ_{max}).

Characterization of complex

The reaction of mefenamic acid and cobalt(II) ions under the described conditions yielded a deep brown complex that was highly light absorbent and therefore could be distinctively qualified via its absorbance in the visible spectral region.

The molar absorptivity of the complex was determined through a calibration equation. Molar ratio of the mefenamic acid-cobalt complex was determined using continuous variations and molar ratio methods. The complex stability constant was calculated by Job's continuous variations method.

Classic optimization

The effects of pH, temperature, agitation rate, and contact time were investigated by varying any one of the process parameters and keeping the other parameters constant. For optimizing each factor, the procedure was the same as that for complex preparation. For evaluation of pH effect, different portions (2 mL) of 50 μg/mL aqueous cobalt(II) solutions in 25 mL volumetric flasks were adjusted to various pH values (5-10) at ambient temperature. Then 2 mL portions of mefenamic acid in DMF (10^{-2} mol/L) were added to each flask with constant stirring within various contact times. The resulting solutions were diluted to the total volume with distilled water and related absorbances were measured. The simultaneous effects of temperature and contact time were also evaluated at 25 and 50 ° C using the same procedure. In each case the temperature was controlled by a digital CRISON thermometer. The procedure was repeated with various agitation rates of 90,120, 150, and 180 rpm to find the best case.

Experimental design

All statistical analysis, modeling, and numerical optimization were performed using Design-Expert software-V.7 (State-Ease, Corp., Minnesota, USA). The 4 variables studied were pH, temperature, agitation rate, and contact time. Factor levels for experimental design were selected based on the results obtained from optimizing the values of factors for the complexation reaction by a one-factor-at-a-time method. Each factor was considered in 3 levels and the levels assigned are given by their actual form in Table 1.

Table 1. Experimental factors with their actual and coded levels.

Factors	Low level (-1)	Medium level (0)	High level (+1)
pH / Factor A	7	8	9
Temperature (°C) / Factor B	20	25	30
Agitation rate (rpm) / Factor C	120	150	180
Contact time (min) / Factor D	4	7	10

A design matrix based on Box-Behnken design including 29 experiments was planned in terms of both actual and coded factor levels. The percent cobalt ion complexation with mefenamic acid reagent was considered as response. The average of 3 replicate measurements was used for each trial in the RSM.

The percentage of removed metal ion solution for each treatment can be calculated using Eq. (1).

$$\textit{Metal binding ability (\%)} = \textit{Metal ion removal}(\%) = (\frac{A_0 - A}{A_0}) \times 100 \quad (1)$$

where A_0 is the initial metal ion absorbance and A represents the final absorbance of metal ion solution.

Results and Discussion

Complex properties

The absorption spectrum of the mefenamic acid-cobalt(II) complex is shown in Figure 3a. The complex obtained shows a relatively wide band with λ_{max}=560 nm. Mefenamic acid in DMF solution shows 3 characteristic bands: at 240, 286, and 352 nm (Figure 3b).

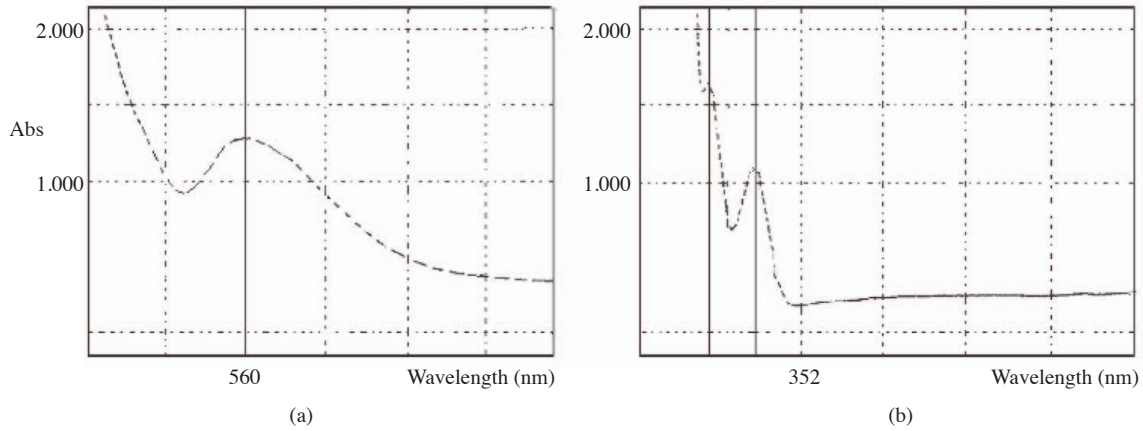

Figure 3. (a) Uv-vis absorbance spectrum of mefenamic acid-cobalt(II) complex, $C_{cobalt(II)} = 7$ ppm (b) Mefenamic acid Uv-vis absorbance spectrum in DMF medium.

The absorption spectrum would be free from any spectral interference and maximum absorbance at 560 nm against a cobalt(II) solution is attributed to the mefenamic acid-cobalt(II) complex.

The molar ratio of metal to ligand was determined by continuous variations and molar ratio methods.[24] Results from the 2 methods were in good agreement and the complex stoichiometric composition was found to be 1:1 (Figures 4 and 5).

In the plot of complex absorbance versus molar ratio of metal, absorbance of the mefenamic acid-cobalt(II) complex passes through a maximum at a molar ratio of 0.5, which confirms that the complex stoichiometry is 1:1.

Job's method of continuous variations is both simple and widely used for the spectrophotometric determination of formulae of metal complexes.[25,26] Generally, the stability constant is an equilibrium constant that expresses the propensity of a substance to form from its component parts. Job's method, under favorable circumstances, can be used to determine the stability constant for a metal-ligand complex, since the deviation of the experimentally determined curve from the extrapolated lines arises from dissociation of the complex. However, a similar deviation may be caused by departures from Beer's law, and so the method is only reliable for moderately adsorbing solutions in which Beer's law applies well.

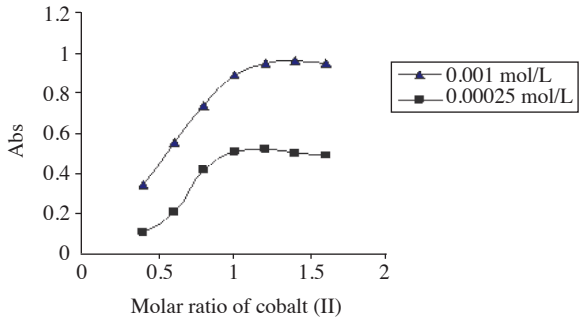

Figure 4. A plot of the Abs. versus mole fraction of cobalt(II) for different concentrations of reactants (10^{-3} mol/L and 2.5×10^{-4} mol/L) in mole-ratio method.

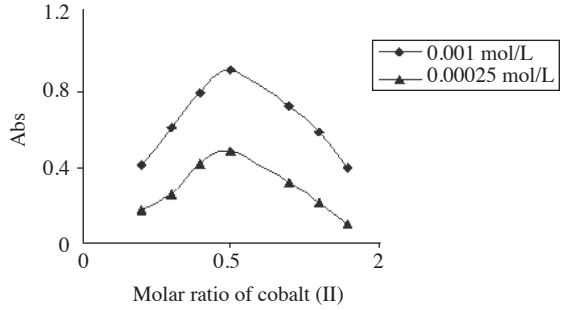

Figure 5. A plot of the Abs. versus $V_M/V_M + V_L$ for 2 different reactant concentrations (10^{-3} mol/L and 2.5×10^{-4} mol/L) in continuous variations method.

The stability constant of the mefenamic acid-cobalt(II) complex was calculated to be 1.076841×10^5 (log K = 5.0321) at ambient temperature (Figure 6). The high value for the stability constant of the mefenamic acid-cobalt(II) complex reveals the significant binding strength of cobalt(II) ions with mefenamic acid. However, the complex possesses high thermodynamic stability at ambient temperature, which denotes the completeness of the related chemical reaction.

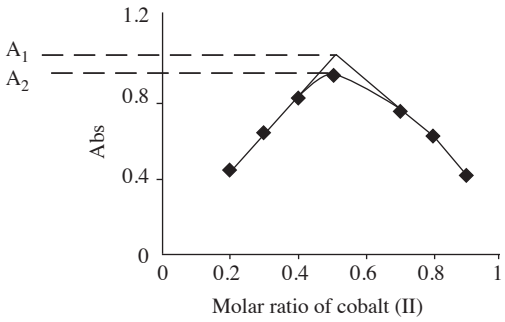

Figure 6. Application of Job's method in determination of mefenamic acid-cobalt(II) complex using 10^{-3}mol/L solutions.

The calibration equation of the mefenamic acid-cobalt(II) complex could be given by

$$Y = 0.1879C + 0.0144 \tag{2}$$

where Y is the absorbance of complex and C is the initial concentration of cobalt(II) ions. The molar absorptivity of the mefenamic acid-cobalt(II) complex was determined to be 1.14×10^4 L/mol.cm. Moreover, the limit of detection was found to be 0.0153 ppm of cobalt(II) concentration.[27]

Classical optimization method

Effect of pH and contact time

As described above, various samples were investigated to optimize pH for the complexation reaction within different contact times. The results are shown in Figure 7.

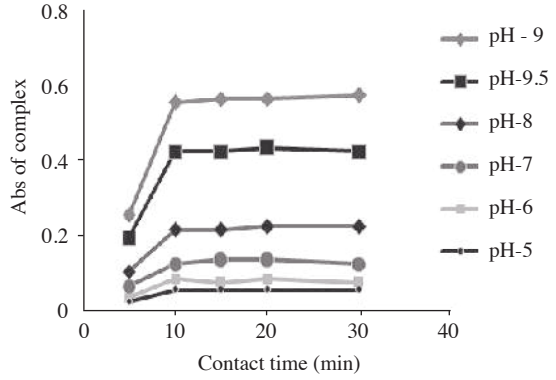

Figure 7. Mefenamic acid-cobalt(II) complex absorbance at various pH values resulting from different contact times, Conditions: 10^{-2} mol/L of drug solution, 50 μg/mL of cobalt(II) solution, agitation rate of 180 rpm and 25 °C.

The results showed that complex formation was intensified in alkaline media. However, the best yield was achieved in pH 9. Equilibrium was attained within 10 min at 25 °C. Therefore, equilibrium could be reached in a short contact time. The pH effect can be well described via dissociation of mefenamic acid in alkaline medium due to the appearance of negative charge and affinity for cobalt(II) ions.

Effect of temperature and contact time

Absorbance of mefenamic acid-cobalt(II) complex was evaluated within various contact times at 25 and 50 °C (Table 2). As seen above, equilibrium was achieved within 10 min, which can be well demonstrated from the flat form of the plot in Figure 8. An increase in temperature from 25 to 50 °C decreased the required contact time from 10 to 5 min and would be desirable to achieve equilibrium, but owing to the possibility of drug hydrolysis at higher temperatures the optimum condition would be considered at 25 °C.[24]

Table 2. Simultaneous effect of temperature and contact time on complex absorbance.

Time (min)	Abs (25 °C) (560 nm)	Abs (50 °C) (560 nm)
5	0.452	0.559
10	0.558	0.562
15	0.561	0.562
20	0.560	0.561
30	0.562	0.562

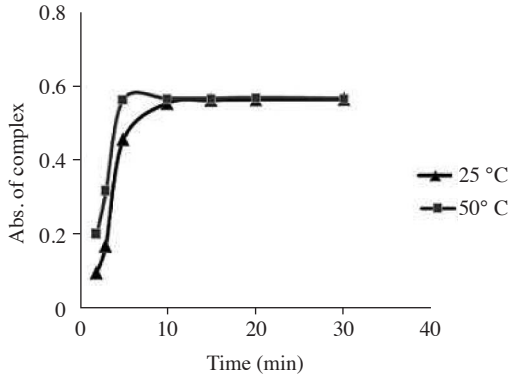

Figure 8. Mefenamic acid-cobalt(II) absorbance as a function of contact time at 25 and 50 °C, Conditions: pH 9, Initial drug concentration of 10^{-2} mol/L, 50 μg/mL of cobalt(II) solution and agitation rate of 180 rpm.

Effect of Agitation rate

Figure 9 shows the absorbance of the mefenamic acid-cobalt(II) complex using different agitation rates.

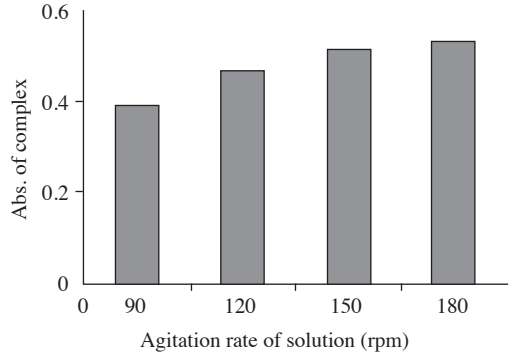

Figure 9. Effect of agitation rate on mefenamic-cobalt(II) complex formation, conditions: pH 9, contact time of 10 min, Initial drug concentration of 10^{-2} mol/L, 50 μg/mL of cobalt(II) solution and temperature of 25 °C.

This diagram revealed that complex formation was not significantly affected by varying stirring rates. However, due to the resulting data, the optimum case was considered to be 180 rpm.

Experimental design

The 4 variables under study, namely pH, temperature, agitation rate, and contact time, were designated as A, B, C, and D, respectively. A Box-Behnken design matrix containing 29 experiments was planned. For each trial, related response (percent cobalt ion binding) was obtained (Table 3).

In the Design-Expert program, the transformation is defined as a mathematical conversion of response values. It is used to satisfy the assumption required for the analysis of variance technique. If the ratio of maximum to minimum response value becomes greater than 10, transformation is usually required. However, for ratios less than 3, the power transformation would have little effect. In the present experimentation, response values range from 31.4 to 76.9, which gives a ratio of 2.44904. Therefore, no transformations were applied.

Table 3. Box-Behnken design with coded factor levels.

Run	A	B (°C)	C (rpm)	D (min)	Result (%)	Run	A	B (°C)	C (rpm)	D (min)	Result (%)
1	0	+1	0	+1	63.7	16	-1	0	0	-1	31.4
2	0	-1	-1	0	51.0	17	+1	0	+1	0	71.7
3	-1	0	0	+1	41.1	18	0	+1	0	-1	54.5
4	+1	+1	0	0	74.2	19	+1	0	+1	+1	76.9
5	-1	-1	0	0	38.1	20	0	0	+1	+1	63.9
6	0	0	0	0	61.5	21	0	+1	-1	0	61.6
7	-1	0	-1	0	39.9	22	+1	0	-1	0	70.9
8	0	0	0	0	61.9	23	-1	0	+1	0	36.3
9	0	-1	0	+1	59.5	24	+1	0	0	-1	61.0
10	0	0	+1	-1	51.2	25	0	-1	+1	0	60.4
11	0	0	-1	-1	50.8	26	+1	-1	0	0	65.4
12	0	0	0	0	62.9	27	0	0	0	0	60.3
13	-1	+1	0	0	39.5	28	0	0	-1	+1	62.6
14	0	-1	0	-1	52.9	29	0	+1	+1	0	63.5
15	0	0	0	0	62.1						

A: pH, B: Temperature, C: Agitation time, D: Contact time, Result: Percent cobalt(II) binding

Box-Behnken design provides fewer runs while the similar 3-level factorial design for 4 factors included 87 experiments. A comparison between the 2 methods reveals the Box-Behnken method to be more economical, convenient, and time efficient. The relative standard deviation (RSD) for 7 replicate measurements was 0.547%.[28,29]

With the Box-Behnken design methodology, major and interaction effects can be easily evaluated. The major effect refers to the effect caused by the varied factor, while the interaction effect is related to the case in which the effect of one factor is dependent on the value of another.[30] The significant factors in the regression model can be estimated by performing analysis of variance (ANOVA). ANOVA for the response surface quadratic model is shown in Table 4.[30]

Regression analysis of the experimental data showed that a quadratic model (values of Prob>F less than 0.0001) best fit the relationship between the response (percent of cobalt(II) complexation) and pH, temperature, agitation rate, and contact time. The Model F-value of 78.44 implied that the model was significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

The result of the experimentation should be a model that will adequately predict the response within the design space. In the statistical output, the lack of fit should not be significant. A small F value and high P value (greater than 0.1) are good in this test. The "Lack of Fit F-value" of 3.34 implies the lack of fit is not significant relative to the pure error.

Values of "Prob > F" less than 0.0500 indicated that the model terms were significant. Values greater than 0.1000 indicate that the model terms are not significant. According to the data, 3 main effects (pH, temperature, and contact time) along with 2 second-order main effects (A^2 and D^2) were significant model terms. Because the model contained significant and non-significant terms (Table 4), it was reduced by

elimination of insignificant terms to achieve a more desirable model (Table 5). Therefore, the new model terms would be A, B, D, A^2, and D^2.

Table 4. ANOVA for response surface quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	P value F Prob > F
Model	3865.39	14	276.10	78.44	< 0.0001
A	2945.71	1	2945.71	838.93	< 0.0001
B	67.69	1	67.69	19.23	0.0007
C	7.75	1	7.75	2.20	0.1938
D	332.88	1	332.88	94.58	< 0.0001
AB	13.69	1	13.69	3.89	0.0723
AC	6.57	1	6.57	1.87	0.2671
AD	5.58	1	5.58	1.59	0.1256
BC	18.92	1	18.92	5.38	0.0384
BD	1.69	1	1.69	0.48	0.5057
CD	0.54	1	0.54	0.15	0.8165
A^2	230.77	1	230.77	65.56	< 0.0001
B^2	9.76	1	9.76	2.77	0.1041
C^2	9.73	1	9.73	2.77	0.0009
D^2	61.45	1	61.45	17.46	0.0013
Residual	49.28	14	3.52	-	-
Lack of fit	44.00	10	4.40	3.34	0.1283
Pure error	-	4	1.32	-	-
Cur total	-	28	-	-	-

Table 5. ANOVA for response surface modified quadratic model

Source	Sum of Squares	df	Mean Square	F Value	P value F Prob > F
Model	3788.92	5	757.78	138.61	< 0.0001
A	3129.87	1	3129.87	572.49	< 0.0001
B	67.69	1	67.69	12.38	0.0018
D	361.90	1	361.90	66.20	< 0.0001
A^2	206.01	1	206.01	37.68	< 0.0001
D^2	45.79	1	45.79	8.38	0.0082
Residual	125.74	23	5.47	-	-
Lack of fit	120.47	19	6.34	4.81	0.0691
Pure error	5.27	4	1.32	-	-
Cur total	3914.67	28	-	-	-

The modification of the model did not affect its adequacy since the correlation coefficient (R^2) and the adjusted correlation coefficient for the reduced model were satisfactory and the predicted correlation coefficient value enhanced (Table 6). Adjusted R^2 is the R-squared adjusted for the number of terms in the model relative to the number of points in the design. Predicted R-squared is a measurement of the amount of variation in new data explained by the model.

Table 6. Values of correlation coefficients (R^2) for the full and modified quadratic models from ANOVA analysis.

Types of coefficient of regression	Full quadratic model	Reduced quadratic model
R^2	0.9874	0.9679
Adjusted R^2	0.9748	0.9609
Predicted R^2	0.9296	0.9484

The model described in terms of coded factors could be shown by final regression equation as

$$R_1 = + 60.19 + 16.15A + 2.38B + 5.49D - 5.46A^2 - 2.58D^2 \quad (3)$$

where A is pH, B is temperature, and D represents the contact time.

The calculated coefficient values in terms of linear, interactions, and quadratic terms are given in Table 7, in which the significant factors can be seen clearly.

Table 7. Calculated quadratic model coefficient values in terms of linear and interactions.

Quadratic model term	Coefficient value	Quadratic model term	Coefficient value
A	+16.15	D^2	-2.58
B	+2.38	AB	+2.15
C	-0.023	AC	-0.47
D	+5.49	AD	+0.88
A^2	-5.46	BC	-0.33
B^2	-0.66	BD	+0.61
C^2	-1.22	CD	-0.30

Term coefficients in Eq. (1) can obviously demonstrate that the ANOVA results as the effect of each variable can be directly attributed to its coefficient value and more effective factors possess higher mathematical coefficients. Since the equation is represented in terms of coded factors, the relative effect of each variable can be determined by comparing the absolute value of its coefficient and its algebraic sign. All single factors under study possessed a positive sign; hence, increasing any one would increase the complexation between 2 reagents. In contrast, second order main effects (A^2 and D^2) had a negative algebraic sign, which indicated a negative effect on complexation reaction. pH of medium (factor A) had the largest effect on the response, followed by contact time (factor D) and temperature (factor B). Increasing pH resulted in better metal binding as a result of hydrolysis in the carboxylic group and producing a negative charged carboxylate site, which tends to chelate with positive cobalt ions. Similarly, an increase in

contact time would lead to more rapid equilibrium attachments and hence more complexation efficiency. The large dependence of reaction yield upon contact time may show the robustness of the method. Increasing temperature would also allow a more thorough reaction. The results were in agreement with those of the classical investigation.

The perturbation plot of cobalt binding ability against all 4 investigated variables implies the contribution of each factor to the complexation reaction (Figure 10). The perturbation plot illustrates percent cobalt(II) complexation as each variable moves from the chosen reference with all other factors held constant at the middle of the design space (the coded zero level).[31]

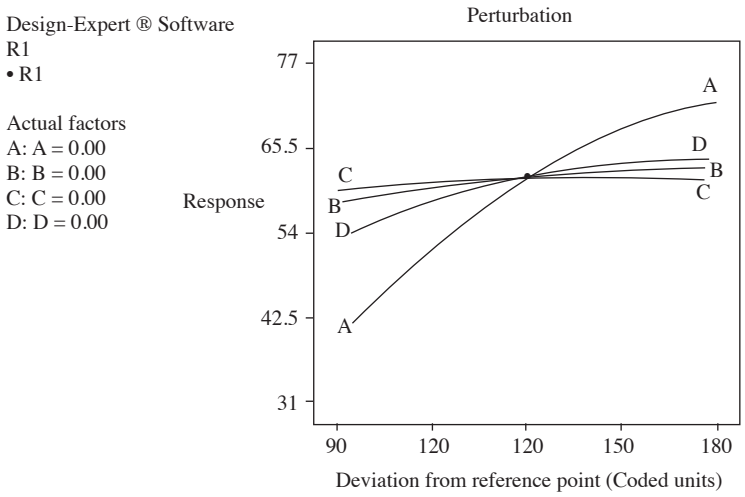

Figure 10. Perturbation plot of cobalt(II) binding ability (R_1) against 4 investigated variables. A represents pH, B is temperature, C is agitation rate, and D represents contact time.

pH (factor A) is shown to have the largest effect on response while agitation rate shows nearly no meaningful effect on the complexation process. The order of effective factors is A > D > B > C.

The optimization by Design-Expert provides a combination of factor levels that simultaneously satisfy the requirements considered for each of the responses and factors. The numerical optimization of Design-Expert is based on the desirability function.[32] The desirability is an objective function that ranges from 0 outside of the limits to 1 at the goal. This function transforms each response value to a desirability index. Each desirability index is described by 3 parameters, which can be defined as goal, lower, and upper. The program allows the desirability index goal parameter to be to one of the options: minimum, maximum, target, in range, or equal to. Once these settings have been defined, the desirability index varies between 0, which shows the worst condition, and 1, which indicates the ideal case. The program looks for the largest overall desirability index and presents a series of solutions that best maximize the desirability index. Table 8 lists the optimization criteria settings applied to optimize the complex formation between mefenamic acid and cobalt ions.

In order to provide an ideal case for complexation between drug and metal ion, the goal for metal binding ability was initially set at maximum. In this way, pH, agitation rate, contact time, and temperature could be targeted equal to their upper limits, with the understanding that in this case better responses would be produced, but we aimed to determine which combination of factors would result in better responses, and so the optimization criteria for factors A, B, C, and D were set in range. Furthermore, there was not a significant difference in responses comparing 2 settings (in range and targeted at upper limits). Other

optimization parameters were set to the default settings of 1 for the weights of lower and upper limits for input data, and 3 for the importance. Importance can be defined as a relative scale that weights each resulting desirability index in the overall desirability product.

Table 8. Settings for numerical process optimization.

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
pH	in range	7	9	1	1	3
Temperature (°C)	in range	20	30	1	1	3
Agitation rate (rpm)	in range	120	180	1	1	3
Contact time (min)	in range	4	10	1	1	3
Percent cobalt(II) binding (%)	maximum	31.4	76.9	1	1	3

Numerical optimization produced 55 optimum solutions with desirability of almost 1.00. The response values produced ranged from 69.3813% (worst case) to 77.9711% (ideal case). The best 10 solutions are sorted in Table 9.

Table 9. Optimum solutions of numerical optimization.

Number	pH	Temperature (°	Agitation rate (rpm)	Contact time (min)	Percent cobalt (II) bilding to mefenamic acid (%)	Desirability
1	8.95	29.5	152	9.49	78.0088	1.00
2	8.99	28.85	165	9.55	77.8265	1.00
3	8.98	28.9	169	9.49	77.8265	1.00
4	9.00	29.4	143	9.04	77.7403	1.00
5	8.92	29.1	166	9.88	77.6807	1.00
6	8.94	28.55	150	9.88	77.6557	1.00
7	8.97	29.95	143	8.71	77.5363	1.00
8	8.97	28.45	163	9.31	77.5639	1.00
9	8.94	28.75	152	9.46	77.4969	1.00
10	8.94	29.5	138	9.34	77.4866	1.00

Table 7 shows that pH ranged from 8.92 to 9.00, temperature from 28.45 to 29.95 °C, agitation rate from 138 to 169 rpm, and contact time from 8.71 to 9.88 min. These results showed that the cobalt-mefenamic acid complex could be formed under various conditions. Selecting the best combination depends on the experimental circumstances.

Conclusion

Mefenamic acid-cobalt(II) complex was prepared in aqueous media. The complex was highly stable under ambient conditions. The classic optimization and response surface methods based on Box-Behnken design

showed that, when multiple variables were involved, studying the system using conventional and classical approaches of studying a process by maintaining other variables involved at constant level would not identify the order of factor effects and combined interactions. Furthermore, the method would be time consuming and require a costly route. Statistical approaches take all of the variables and their interactions into consideration simultaneously. Therefore, developing mathematical models describing the relationship between the response and independent variables in which the significance of individual factors and multifactor interactions can be determined would be possible. A quadratic model showed a good fit with the experimental data (R^2 = 0.9609). According to the results obtained, mefenamic acid can be considered a suitable chelating agent for cobalt ions that may provide better or different pharmacological profiles than that of the free drug.

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